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Vascular Biology of arterial aneurysms

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Abstract:

Objective: This review aims to analyze biomolecular and cellular events responsible for arterial aneurysm formation with particular attention to vascular remodeling that determines the initiation and the progression of arterial aneurysm, till rupture. **Methods:** this review was conducted searching libraries such as Web of Science, Scopus, ScienceDirect and Medline. Used keywords with various combinations were: “arterial aneurysms”, “biology”, “genetics”, “proteomics”, “molecular”, “pathophysiology” and extracellular matrix”. **Results:** there are several genetic alterations responsible of syndromic and non-syndromic disease that predispose to aneurysm formation. ECM imbalance, mainly due to the alteration of vascular smooth muscle cells (VSMCs) homeostasis, overexpression of metalloproteinases (MPs) and cytokines activation, determines weakness of the arterial wall that dilates thus causing aneurysmal disease. Altered mechanotransduction in the ECM may also trigger and sustain anomalous cellular and biochemical signaling. Different cell population such as VSMCs, macrophages, perivascular adipose tissue (PVAT) cells, vascular wall resident stem cells (VWRSCs) are all involved at different levels. **Conclusions:** Improving knowledge in vascular biology may help researchers and physicians in better targeting aneurysmal disease in order to better prevent and better treat such important disease.

1. Introduction

An arterial aneurysm is defined a permanent localized dilatation of an artery with at least a 50% increase in diameter compared to the normal value of the adjacent non-affected vessel segment (or ≥ 1.5 times, or ≥ 150% the normal diameter).[1-5] Aneurysms can be classified according to several factors. In fact, *by nature*, they can be distinguished in *true* aneurysms when all the three layers of the arterial wall are involved in aneurysm formation or in *false* aneurysms (or pseudoaneurysms), secondary to various arterial injuries, in which not all the vessel layers are involved, and the condition mimics the presence of an aneurysm; by *morphology*, they can be distinguished in *saccular* when dilation is localized in a specific part of the vessel diameter or *fusiform* when all diameter is involved; by location, they can be distinguished in *central* (aortic), *peripheral* (carotid, subclavian, iliac, popliteal, etc.), *visceral* (splenic, hepatic, coeliac trunk, mesenteric, renal, etc.), and *intracranial*; by *etiology* they can be distinguished in *degenerative* or *idiopathic* (often of atherosclerotic origin), *infective* (of infectious origin, e.g. syphilis), *inflammatory* (due to arteritis such as Takayasu disease).[2] Generally, an arterial aneurysm if not treated, will progressively grow, during time, with increasing risk of rupture. The consequence of rupture varies with the location of the aneurysm and the extent of the related blood loss.[6] The epidemiology of arterial aneurysms varies according to location. The Aorta is the most frequent locations of aneurysmal disease and aortic aneurysms (AAs) have a prevalence that ranges between 1.3% and 18% for abdominal aortic aneurysms (AAAs)[7-8] and with a prevalence of 0.16% for thoracic aortic aneurysms (TAAs).[9] Moreover, thoracoabdominal aortic aneurysms (TAAAs), that involve both the descending thoracic and abdominal aorta, account for 5% to 10% of all TAAs.[10] Peripheral artery aneurysms are represented mainly by popliteal artery aneurysms (PAA) with a prevalence < 1%[11] and isolated iliac artery aneurysms with a prevalence < 0.03%[12] as femoral artery aneurysms are quite rare and often associated with other peripheral or aortic aneurysms.[13] Visceral artery aneurysms are also quite rare accounting for a prevalence < 0.2%.[14] Intracranial aneurysms (IAs) are quite common accounting for a prevalence of 1-2% in the general population.[15]

Genetic, biomolecular and cellular events responsible for arterial aneurysm formation are quite complex, and finally determine important pathological changes in the anatomy and function of the vessel wall with arterial remodeling.[16-17]

Deciphering biological mechanisms of arterial aneurysms could potentially help in optimizing patients' management and related outcomes.[18] This review article aims to summarize the most updated information regarding arterial aneurysms highlighting the issues that can improve the understanding of pathophysiologic mechanisms.

To conduct this review, searched libraries were Web of Science, Scopus, ScienceDirect and Medline. Used keywords with various combinations were: "arterial aneurysms", "biology", "genetics", "proteomics", "molecular", "pathophysiology" and extracellular matrix".

2. Biology of the arterial wall

The arterial wall consists of three layers: intima (the inner layer), media (the middle layer) and adventitia (the outer layer).

The intima layer includes endothelial cells (ECs) that lie on the basement membrane (BM) and is supported by internal elastic lamina (IEL). Intima layer covers the luminal surface of the vessel. ECs produce several vasoactive molecules with vasodilating and anticoagulative actions and are the main regulators of vascular homeostasis. [19] BM is a thin structure of extracellular matrix (ECM) components that serves as a cellular adhesion site, and also as a barrier and a signaling center for the intima. [20]

The media layer is situated beyond the IEL and is composed of vascular smooth muscle cells (VSMCs) concentrically positioned. This layer is characterized of important elements of ECM such as collagen, elastin, proteoglycans, cadherins, and integrins. In fact, this layer is the most represented part of the entire vessel mass and have also an important role in supporting the structure of the artery, securing adequate elasticity and strength to the artery wall. [19,21-23] In fact, VSMCs adhere to the ECM

through integrins on cell surface, and integrin receptors bind to microfibrils connected to elastin fibers, thus forming the VSMC elastin-contractile unit that modulate VSMCs contractive activity in response to blood flow. Furthermore, integrins on cell surface may interact with other VSMCs and with ECM proteins such as metalloproteinases (MPs) and transforming growth factor β (TGFβ). The interaction of TGFβ and the glycoprotein fibrillin, secreted into the ECM by fibroblasts, is pivotal to the assembly of microfibrils. [22-23] Thus, VSMCs and their interactions, are involved in several pathological processes related to arterial remodeling, including aneurysms[24-55].

The adventitia layer is mainly composed of abundant collagen, fibroblast, mast cells, macrophages, T-cells, B-cells, dendritic cells, lymphatic vessels and autonomic nerves. It is implicated in surveillance for foreign antigens and in the communication between ECs and (VSMCs) [19,26].

Perivascular adipose tissue (PVAT) surrounds the adventitia layer of large blood vessels, except the intracranial vessels, and is even considered the fourth layer of the blood vessel wall (tunica adiposa) for its role in vascular biology, vascular homeostasis and several vascular diseases including aneurysms. [27-29]

Vascular wall resident stem cells (VWRSCs), situated in all the aforementioned three layers of the artery, is a group of undifferentiated cells that upon specific cellular demands, may turn in specific vascular wall cells, thus playing an important role in physiology and in disease conditions, including aneurysms. [30-31]

3. The genetic influence

This section discusses the genetic influence in AAs, and IAs for which, due to their major prevalence in aneurysmal disease, there is pertinent and robust literature.

The effects of gene alteration in aneurysmal disease have been classified into two main groups: syndrome disease in which gene mutations that predispose to aneurysm formation are also associated

to abnormalities of other organs and body systems, and non-syndromic disease in which gene mutations determine only aneurysms formation without other phenotypic clinical manifestations. [32]

Among syndromic disease, mutations in fibrillin-1 (*FBN1*) gene identify Marfan Syndrome (MS), an autosomal dominant heritable disorder of the connective tissue with cardiovascular, pulmonary, skeletal, and ocular clinical manifestations. Among cardiovascular problems, TAAs are frequent. *FBN1* gene encodes for the glycoprotein fibrillin that has biomechanical functions for the media layer of vessels, interacting with TGFβ for microfibrils assembly. Most *FBN1* alterations are represented by point mutations that cause disorganizations of fibrils structure. Moreover, fibrillin is also unable to adequately saturate TGFβ binding with subsequent elevated free TGFβ with disturbed signaling activity[23] that contributes to aneurysms formation[33]. The alterations of microfibrils determine the impossibility to bind elastin fibers that trigger elastin fragmentation through the activity of MPs, with further ECM degeneration. Furthermore, if for the aforementioned mechanisms, VSMCs result no longer adequately linked to ECM in a useless attempt of repair they turn into apoptotic phase with cellular death. All these mechanisms lead to loss of mechanical integrity with medial degeneration.[23]

Mutations of the genes that encodes for the receptors 1 and 2 of transforming growth factor Beta, respectively *TGFBR1* and *TGFBR2*, are responsible of Loeys-Dietz-syndrome (LDS), an autosomal dominant syndrome, that causes connective tissue alterations with vascular disease including TAAs, skeletal anomalies, craniofacial abnormalities and cutaneous alterations. [23,32] Alterations of TGFβR1 and TGFβR2 lead to disturbed signaling activity of TGFβ similarly to MS. [23]

Mutations of the gene collagen type III alpha 1 chain (*COL3A1*) is associated with the vascular type of Ehlers-Danlos syndrome, an autosomal dominant heritable disorder, that affect the structure of type III collagen, a fundamental protein of the artery medial layer, synthetized by VSMCs. For this alteration vascular rupture is very frequent, even without aneurysm. [23,32]

Alterations in the aforementioned genes may also be found in non-syndromic disease also with other genes related to VSMCs function and metabolism and adhesion to ECM, that can predispose to

TAAs such as smooth muscle α-actin 2 (*ACTA2*), myosin light chain kinase (*MYLK*), myosin heavy chain 11 (*MYH11*), lysyl oxidase (*LOX*), protein kinase cGMP-dependent type 1 (*PRKG1*), mothers against decapentaplegic homolog 3 (*SMAD3*). [22,32]

Chen et al[34] explored possible druggable genes in the development of AAs and performed a large-scale Mendelian randomization analysis using genome-wide association study (GWAS) datasets, drug genome and gene expression data and found that Urokinase-type plasminogen activator (*PLAU*) gene, and proteasome 20S subunit alpha 4 (*PSMA4*) can be effectively targeted. In particular, *PLAU* encodes a protein, U-plasminogen activator (uPA) that converts plasminogen to plasmin, a protein that is pivotal in controlling proteolytic events of collagen type IV and fibronectin in the ECM. Protein uPA regulates activities of some MPs, in particular matrix metalloproteinase (MMP)-9 and MMP-12 and interacts also with TGFβ and vascular endothelial growth factor (VEGF). Moreover, uPA can trigger vascular inflammation stimulating the actions of several cytokines, and MMPs (MMP-2 and MMP-9). PSM4 encodes a proteasome subunit related regulation of several cellular and ECM processes such as inflammation, MMP activity, elastin degradation, VSMCs homeostasis, and apoptosis. *PLAU*, and *PSMA4* seem to be two possible drug targets that may reduce AAs risk.

GWAS identified single-nucleotide polymorphisms of several genes that seem to be associated with AAAs. In particular, a variant (rs2836411) of erythroblast transformation-specific (ETS)-related gene (*ERG*), that physiologically controls vascular development and angiogenesis, seems to trigger specific pathways with AAA development such as matrix remodeling and endothelial cell activation. Variant of *MPs* and tissue inhibitors of matrix metalloproteinases (*TIMPs*) genes, of lipid metabolisms genes, and of inflammatory cytokines receptors genes such as Interleukin (IL) 6 receptor (R) (*IL6R*) seem to be associated with AAAs. [35-36]

Syndromic IAs are represented by autosomal dominant polycystic kidney disease (ADPKD) [15] which is characterized also by manifestations, such as great kidneys cysts, arterial hypertension, liver cysts, and cardiac valvopathy[37]; multiple endocrine neoplasia type I (MEN1) [15], which is characterized also by parathyroid, enteropancreatic, pituitary and adrenal glands tumors[38] ; hereditary

hemorrhagic telangiectasia (HHT) [15] which is characterized also by gastrointestinal, pulmonary and hepatic lesions[39]; neurofibromatosis type I[15] which is characterized also by cardiac, dermatologic, gastrointestinal, and orthopedic lesions; vascular type of Ehlers-Danlos syndrome and Marfan's syndrome[15] that were cited previously in this text.

Non syndromic IAs are generally due genetic alterations that involves VSMCs as they have a fundamental role in IAs development and rupture. Specifically, VSMCs phenotypic modulation from a contractile to a proinflammatory state is an important step in IA formation and it is controlled by several genes. In particular, *MYH11*, Calponin 1 (*CNN1*), Myocardin (*MYOCD*), smooth muscle α-actin 1 (*ACTA1*), Leiomodin 1 (*LMOD1*) are genes that confers VMSCs contractile and structural properties, whereas Complement C1q B Chain (*C1QB*), Complement C3a Receptor 1 (*C3AR1*), V-set and immunoglobulin domain containing 4 (*VSIG4*) are genes related to the complement system and therefore to inflammation[40].

Furthermore, common genetic pathways, related to five chromosomal regions, have been postulated both for AAs and IAs. In particular, 3p24-25 and 5q for TAAs and IAs; 4q32-34 and 19q for AAAs and IAs; 11q24 for TAAs, AAAs and IAs [41].

The role of epigenetics has been widely studied in AAAs. Smoking, a common risk factor found in about 80% of AAA patients, seems to induce expression of several genes, such as lipoxygenase-5 (*5-LO*), involved in the activation of inflammatory pathways that lead to AAA formation. Inflammation is also responsible of inducing epigenetic mechanisms, such as hyper-methylation of gene promoter regions of several pro-inflammatory cytokines such as IL-6 and tumor necrosis factor-α (TNF-α), thus chronically maintaining inflammation processes that stimulate MPs and other proteins responsible of ECM degradation and VSMCs apoptosis. Moreover, IL-6 may sustain epigenetic changes maintaining methylation on VSMCs by the regulation of DNA methyltransferase 1 (*DNMT1*) gene[42].

In the context of DNA methylation, hypomethylation of the gene SET (Suppressor of variegation, Enhancer of Zeste, Trithorax) and MYND (Myeloid-Nervy-DEAF1) domain-containing 2 (*SMYD2*),

a VSMCs gene involved in myofibril organization, determines chronic inflammation and alteration of the arterial wall that trigger AAA formation[36].

Moreover, histone modification, mediated by various histone deacetylases (HDACs), may alter gene expression of several *MMPs* such as *MMP2* and *MMP9* with consequent excessive proteolysis and degradation of ECM. In this context, several evidence suggest that epigenetic modifications may be targeted by HDAC inhibitors and DNA methylation inhibitors, and in the next future epigenetic therapy for AAAs may also be sustainable.[36,42]

Several microRNAs (miRNAs) seem to promote VSMCs proliferation and/or alteration of TGFβ signaling such as miR-146a and miR-26a, while some other miRNAs, such as miR-143 and miR-145, seem to increase contractility of VSMCs, protecting from AAA formation, and, interestingly, their expression is found decreased in AAA patients. There is also a role of some long noncoding RNAs (lncRNAs) in AAA formation, as they could impair VSMCs proliferation, or inducing anomalous apoptosis in these cells.[36]

Roychowdhury et al [43] identified a locus in the intronic region of transcription factor 7 like 2 (*TCF7L2)* that is associated with TAA, and this is the first locus identified for TAA via GWAS. TCF7L2 seem to be involved in VSMCs apoptosis, a key factor in TAA.

Bakker et al [44], via GWAS, showed that in IAs, a SNP-based heritability of 21.6% is present, explaining over half of the total heritability in this context. They also showed that the majority of IAs heritability is of polygenic nature. They also identified ECs as key cells type in IAs pathophysiology.

4. Extracellular matrix: biochemical and biomechanical imbalance in aneurysm formation

ECM, a complex biopolymer network, is a major component of most human organs, and is a fundamental supporting element of the vessel wall and is also pivotal in intercellular signaling. In the vessel wall, the main components of ECM are represented by elastic fibers that are related to elastin, collagen and microfibrils. Elastic fibers are also important in vessel mechanotransduction processes,

during which mechanical forces from blood flow on the vessel wall are converted in biochemical signals. Also, the control of collagen biosynthesis and degradation is important for vessel wall homeostasis. The physical, geometrical and mechanical properties of the ECM are critical to physiological processes of the vessel wall. In fact, alterations of these processes in the artery wall can lead to the development of several arterial disease, including aneurysms. [45-47]

The enhanced ECM degeneration is due to the alterations of several families of proteases. In particular, MPs are enzymes, that, together with their endogenous tissue inhibitors of metalloproteinases (TIMPs) regulate extracellular structural proteins and consequent tissue remodeling. There are three main families of MPs, "matrix metalloproteinase" (MMP) family, "a disintegrin and metalloprotease" (ADAM) family, and "a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) family. MMPs, are calcium-dependent endopeptidases characterized by 3-histidine zinc-binding motif at the catalytic site and a methionine residue under the zinc active site (Met Turn). These proteases are synthesized as pre-pro-enzymes and secreted as inactive pro-MMPs activated by disruption of cysteine-zinc interaction of the cysteine switch and the removal of pro-peptide. MMPs are secreted by several cell type like neutrophils, macrophages, VSMCs and ECs, and are induced by several molecules such as cytokines and growth factors including IL-1a and b, IL-2, IL-17, insulin like growth factor-1, Epitelial Growth Factor (EGF), transforming growth factor beta (TGF-β), Extracellular Matrix-Metalloproteinase Inducer (EMMPRIN), also known as CD147, and tumor necrosis factor alpha (TNF-α). MMP-2, MMP-9, also known as gelatinases, are the most important MMP members in the remodeling of the ECM and subsequent release of angiogenic factors contributing to the migration of VSMCs from the medial vascular layer to the intimal layer. These proteases, degrade elastin inducing elastosis and inflammation with destruction of all major ECM components with subsequent excessive vessel distensibility, and finally arterial rupture. Moreover, MMP-9, in inflammatory context, is positively modulated by a protein expressed by activated neutrophils, known as neutrophil gelatinase-associated lipocalin (NGAL). Once formed, the NGAL/MMP-9 complex, protects MMP-9 from proteolytic

degradation maintaining for a longer time its activity. In fact, NGAL denotes leukocyte activation, and in arterial aneurysms its levels increase with aneurysmal expansion, thus emphasizing the role of inflammation in disease progression. [48-56]

Several evidence showed a significant correlation between age, median size of different types of aneurysms (intracranial, central, peripheral), rupture, outcome after surgical procedures, and plasma levels of MMP-9 and NGAL, thus allowing these latter, to be considered as biomarkers to predict aneurysmal rupture. [57-60]

Considering gelatinases, several evidence showed that MMP-2 is mainly derived from VSMCs and fibroblasts, and only in part by macrophages, whereas MMP-9 is mainly secreted by macrophages and in part by neutrophils. Acting together, these gelatinases seem to enter in the regulation of several pathways leading to inflammation involving several molecules such as TGF-β, tumor necrosis alpha (TNF-α), interleukin 1 beta (IL-1β), monocyte chemoattractant protein 1 (MCP-1), and reactive oxygen species (ROS). [61]

Furthermore, MMP-12, mainly expressed in macrophages, is directly involved in the degeneration of elastic fibers, also by degrading tropoelastin, a precursor of elastin, that it triggered during ECM damage. It is also related to aneurysm growth and macrophage recruitment. [62-63]

On the other hand, MMP-3, also known Stromelysin-1, and MMP-8, also known as neutrophil collagenase, can digest primarily the other pivotal structural protein of ECM of the vessel wall, collagen, and their expression have been found elevated in immunohistochemical studies in aneurysmatic tissues, correlating to aneurysm expansion. [64-65]

A recent study evaluated in ascending aortic aneurysms the relationship between shear stress and circulating plasma levels of MMP-1, MMP-2 and TIMP-1, documenting the role of these proteins in the imbalance of mechanotransduction pathways that causes subsequent anomalous vascular remodeling. [66]

ADAMs and ADAMTSs have similar structure with a pro-domain, and a metalloproteinase, disintegrin and cysteine-rich domain. ADAMs have also a transmembrane domain, ADAMTs lack

the transmembrane domain but have a thrombospondin motif. Both ADAM and ADAMTS proteins are involved not only in the regulation of ECM structural proteins of the vessel wall, but all also in the modulation of several biological processes such as cell-associated proteins mechanisms, growth factors regulation, and cytokines triggering.[67] There are several solid evidence for the role of ADAM-17 in aneurysmal disease. In fact, it was shown that this protease can induce VSMCs. And macrophages anomalous phenotypic switching causing matrix degradation, inflammation, and VSMCs apoptosis with subsequent vascular remodeling leading to aneurysm formation. ADAM-10 and ADAM-17 have been found upregulated in AAA intraluminal thrombus, and this condition highlight their role in macrophage activation with chronic inflammation and proteolytic activity. Other evidence postulated a role also for ADAM-8, -9, -12, and -15 but the exact mechanisms are still little known. [68] Altered expression of ADAMTS proteins, such as ADAMTS-1, -4, and -5 seem to relate to aneurysmal disease. In particular, these members of ADAMTS family can degrade ECM proteoglycans, such as versican, facilitating macrophage invasion, thus triggering inflammation. [50,69-71]

The catalytic activities of the various MP families are tightly regulated by tissue inhibitors of TIMPs, which consists of 4 members TIMP-1, -2, -3 and -4 that can inhibit theoretically all MP families but actually the in-vivo inhibition is characterized by different ranges of specificity and efficiency for each member of MP family. In particular, TIMPs have a broad inhibitory action on most MMP family members, but specific inhibitory effects on ADAMs family members. For example, while TIMP-1 and TIMP-3 can suppress the ADAM-10 activity, none TIMPs have effect on ADAM-8, -9 and -19. Furthermore, TIMP-3 can specifically inhibit ADAM-17, ADAMTS-4 and -5. Interestingly, the ratios of MP families and TIMPs are crucial for the maintenance of the normal architecture of the ECM and when this ratio is affected ECM imbalance could lead to weakness of the arterial wall and subsequent aneurysm formation. Furthermore, there are also cellular effects of TIMPs activity. In particular, TIMP-1 might also reduce VSMCs and macrophages migration in arterial wall thus indirectly preventing MMPs release. [50,67,72-74]

In the ECM a group of nonstructural proteins, called matricellular proteins, have also been related to aneurysmal disease due to their property to link cellular signaling with biochemical environment of ECM, regulating cell-matrix interactions. These proteins are classified into seven families, and in particular, thrombospondins (TSPs), small integrin-binding ligand N-linked glycoprotein (SIBLING), tenascins (TNs), centralized coordination network (CCN), Gla protein, short fibulins (SFs), and secreted protein acidic and rich in cysteine (SPARC). TSP-1 serum levels seem to be negatively associated with AAAs and a role in regulating MMP-9 and VSMCs apoptosis has been postulated for this protein. TSP-2 may promote VSMCs apoptosis and inflammation by the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). TSP-4 is involved in TGFβ signaling and related to aneurysmal disease, but its specific role remains to be clarified. [75-80] Osteopontin, member of SIBLING family, seems to be related through its proinflammatory actions (neutrophil activation and macrophage infiltration) and induction of VSMCs autophagic processes, to AAA formation. [75,81-82] Tenascin C (TN-C) is activated in VSMCs by cytokines, growth factors, and wall stress and in TAA and AAA can trigger inflammatory and proteolytic effects.[75,83-84] Connective tissue growth factor (CTGF)/ CCN2 has been related to VSMCs phenotype switching contributing to AAA formation. [75,85] Amongst Gla family, periostin seems to activate MMP-2 and MCP-1 expression, in response to vessel wall stress triggering inflammation sustaining aneurysmal formation.[75,86-88] Deficiency of fibulin-4 has been related to loss of contractile phenotype of VSMCs in TAAs but its exact role in aneurysmal disease remains to be elucidated.[75,89] Among, SPARC family, Testican-2 due to its metalloprotease-regulating properties and to its ability to induce VSMCs phenotype switching may trigger artery wall dilation. [75,90-91]

Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) deficiency in mice seems to be related to reduction in fibroblasts and myofibroblasts content and the presence of thinner adventitial collagen determining an increase of ruptured AAA. LOX-1 is also involved in IL-1β production and ECM breakdown triggering specific inflammatory pathways. [92-93]

Lysyl oxidase (LOX), is a copper-binding enzyme that cross-links elastin and collagen and is an important key matrix-modifying enzyme that has been demonstrated to significantly affect structural abnormality and dysfunction of the vessel wall. Alterations of LOX regulation may determine elastase and aggrecan accumulation in the ECM with further disruption of the incompetent elastic fibers causing artery dilation, resulting in arterial aneurysms. [94-95]

Table 2 shows the most studied proteins related to aneurysmal disease for which there is solid evidence in the current literature.

ECM support vessel structure also modulating mechanotranduction. In particular, mechanical stimuli, coming from the vascular microenvironment, due mainly to shear stress and blood pressure, are sensed by mechano-receptors, such as integrins, and converted in biochemical signals triggering cells specific responses. Furthermore, modifications in ECM composition, both due qualitative and quantitative changes, can alter mechanical properties of the artery, thus activating biomolecules signaling that will affect cell homeostasis, such as VMSCs phenotypic changes, thus triggering several vascular diseases, including aneurysms. [96-99]

5. Cellular mechanisms in aneurysms formation

Arterial wall weakening in aneurysmal disease is mainly due to the loss of VSMCs within the media layer, substantially due to apoptosis. VSMCs are pivotal for ensuring structural and functional properties of the artery wall and for several ECM proteins synthesis. VSMCs have the ability to adapt to environmental mechanical stimuli switching between a contractile and a synthetic phenotype. The contractile phenotype confers these cells more differentiated and functional features with the upregulation of VSMCs specific contractile proteins, such as smooth muscle 22alpha (SM22alpha) and alpha smooth muscle actin (αSMA. On the other hand, the synthetic phenotype, downregulate contractile proteins expression and confers these cells proliferative and secretive properties with elevated synthesis of proteases that degrading ECM facilitate VSMCs migration. In addition, VSMCs with synthetic phenotype can secrete extracellular vesicles (EVs), lipid bilayer bound particles, that

contains proteases and other molecules that enhance local inflammation. Ultimately, with the upregulation of osteopontin expression, VSMCs turn into an inflammatory phenotype leading to apoptosis. [100,102]

Macrophage infiltration is one of the most important hallmarks of aneurysmal disease, as these cells are involved in MPs and cytokines synthesis, and similarly to VSMCs are able to switch to different phenotypes related to both inflammatory and reparative actions. Moreover, macrophages can directly influence VSMCs activity through macrophage-derived netrin-1 protein. Considering inflammatory processes, characteristic of aneurysmal disease also T cells and B lymphocytes are involved. In particular, T cells exert a double role, on one hand they sustain chronic inflammation, on the other hand, by their regulatory T cells subpopulation protect against aneurysmal disease for the secretion of IL-10 with anti-inflammatory properties. The role of B-cells is more complex, as different B cell subtypes (B1 and B2) may exert several and even opposite effects in aneurysmal disease. [103]

PVAT has inflammatory properties, recruiting also macrophages and T-cells, that can induce phenotypic changes on cell population related to aneurysmal disease. Moreover, there is evidence of elevated adipocyte accumulation in tissues of ruptured aneurysms ant this accounts for a role of PVAT in progression and rupture of arterial aneurysms.[27-29]

Furthermore, VWRSCs, in presence of ECM imbalance, may on one hand promote vascular repair differentiating in VSMCs and fibroblasts, but on the other hand especially under the influence of MPs, may differentiate into inflammatory cells contributing to aneurysm formation and progression, but the exact role of VWRSCs remains not fully understood. [31]

Figure 1 summarizes the main pathophysiologic steps of aneurysm formation considering what have been showed in all the sections of this paper.

7. Conclusions

In conclusion, vascular biology of aneurysmal disease, is a complex field that includes the study of arterial wall composition, genetic factors, and a multitude of elements that compose ECM that are

related to chronic inflammation, altered protease expression, imbalanced mechanotransduction, and phenotypic switching of several cell population that ultimately lead to vascular remodeling. These pathological processes determine aneurysm initiation, progression, and rupture. Most of the molecules that we showed in this article have been tested to serve as biomarkers, both in pre-clinical and clinical studies in order to predict the susceptibility to disease or to track the outcomes of medical, surgical and endovascular treatments, but to date no proposed biomarker turned effectively into clinical application in this field. Further investigation will identify more molecules, cells and mechanisms involved in aneurysmal disease that may definitely help identify novel specific biological targets for the prevention and the management of aneurysmal disease.

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Tables legends:

Table 1. Main genes and related implications in aneurysm development.
Table 2. More relevant proteins related to ECM imbalance in aneurysm development.

Figures legends:

Fig. 1. Main pathophysiologic steps of aneurysm formation.

Footnote to fig. 1: FBN1 : fibrillin-1; TGFBR1 : transforming growth factor Beta 1; TGFBR2 : transforming growth factor Beta 2 ; MMPs : Matrix metalloproteinase; TIMPs : Tissue inhibitor of metalloproteinase; *COL3A1*: collagen type III alpha 1 chain; *ACTA1*: smooth muscle α-actin 1; *ACTA2*: smooth muscle α-actin 2; *MYLK*: myosin light chain kinase; *MYH11*: myosin heavy chain 11; *LOX*: lysyl oxidase; *PRKG1*: protein kinase cGMP-dependent type 1; *SMAD3*: mothers against decapentaplegic homolog 3; *PLAU :* Urokinase-type plasminogen activator*; PSMA4 :* proteasome 20S subunit alpha 4 *; ERG :* erythroblast transformation-specific (ETS)-related gene*; IL6R:* interleukin 6 receptor*; CNN1:* Calponin 1 *; MYOCD:* myocardin *; LMOD1:* leiomodin 1; *C1QB:* Complement C1q B Chain *; C3AR1: ; VSIG4:* V-set and immunoglobulin domain containing 4 *; 5-LO:* lipoxygenase-5 *; SMYD2:* SET (Suppressor of variegation, Enhancer of Zeste, Trithorax) and MYND (Myeloid-Nervy-DEAF1) domain-containing 2; ADAM : a disintegrin and metalloproteinases ; ADAMTS : a disintegrin and metalloproteinases with thrombospondin motifs ;

NGAL : neutrophil gelatinase-associated lipocalin; TSPs : thrombospondins ; SIBLINGs : small integrin-binding ligand N-linked glycoproteins ; TNs : tensascins ; CCNs : centralized coordination network proteins ; SPARCs : secreted proteins acidic and rich in cysteine ; EVs : extracellular vesicles ; PVAT : Perivascular adipose tissue; VWRSCs : Vascular wall resident stem cells.

Table 1. Main genes and related implications in aneurysm development.

Gene Alteration or Polymorphism	VSMCs functions	ECM alteration	Implication in Chronic Inflammation
FBN1	X	X	
TGFBR1	X	X	
TGFBR2	X	X	
MMPs		X	X
TIMPs		X	X
COL3A1	X	X	
ACTA1	X	X	X
ACTA2	X	X	
MYLK	X	X	
MYH11	X	X	
LOX	X	X	
PRKG1	X	X	
SMAD3	X	X	
PLAU	X	X	X
PSMA4	X	X	X
ERG		X	X
IL6R			X
CNN1	X	X	
MYOCD	X	X	
LMOD1	X	X	
C1QB	X		X

C3AR1	X	X
VSIG4	X	X
5-LO	X	X
SMYD2	X	X
TCF7L2	X	

Footnote. *FBN1*: fibrillin-1; *TGFBR1*: transforming growth factor Beta 1; *TGFBR2*: transforming growth factor Beta 2; *MMPs:* matrix metalloproteinases*; TIMPs*: tissue inhibitor of matrix metalloproteinases; *COL3A1*: collagen type III alpha 1 chain; *ACTA1*: smooth muscle α-actin 1; *ACTA2*: smooth muscle α-actin 2; *MYLK*: myosin light chain kinase; *MYH11*: myosin heavy chain 11; *LOX*: lysyl oxidase; *PRKG1*: protein kinase cGMP-dependent type 1; *SMAD3*: mothers against decapentaplegic homolog 3; *PLAU :* Urokinase-type plasminogen activator*; PSMA4 :* proteasome 20S subunit alpha 4 *; ERG :* erythroblast transformation-specific (ETS)-related gene*; IL6R:* interleukin 6 receptor*; CNN1:* Calponin 1 *; MYOCD:* myocardin *; LMOD1:* leiomodin 1; *C1QB:* Complement C1q B Chain *; C3AR1: ; VSIG4:* V-set and immunoglobulin domain containing 4 *; 5-LO:* lipoxygenase-5 *; SMYD2:* SET (Suppressor of variegation, Enhancer of Zeste, Trithorax) and MYND (Myeloid-Nervy-DEAF1) domain-containing 2; *TCF7L2 :* transcription factor 7 like 2.

Table 2. More relevant proteins related to ECM imbalance in aneurysm development.

Proteins	Actions/Effects
MMP-1	Altered mechanotransduction.
MMP-2	Altered mechanotransduction, migration of VSMVs, elastosis, recruitment of cytokines, chronic inflammation.
MMP-9	migration of VSMVs, elastin degradation, recruitment of cytokines, chronic inflammation, aneurysm growth, aneurysm rupture.
MMP-3, -8	Collagen degradation.
MMP-12	Tropoelastin degradation, macrophage recruitment.
NGAL	Protection of MMP-9 from proteolytic degradation, aneurysm growth, aneurysm rupture.
ADAM-10	Macrophage activation, chronic inflammation.
ADAM-17	Macrophage activation, chronic inflammation, VSMCs apoptosis.
TIMP-1	MP inhibition, reduction of VSMCs and macrophages migration.
TSP-1	Activation of MMP-9, induction of VSMCs apoptosis.
TSP-2	Chronic inflammation, induction of VSMCs apoptosis.
TSP-4	Alteration of TGFβ signaling.
Osteopontin	Neutrophil activation, macrophage infiltration.
TN-C	Chronic inflammation, proteolytic effects.
CTGF/CCN2	VSMCs phenotype switching.
Fibulin-4	Loss of contractile phenotype of VSMCs.

Testican-2	VSMCs phenotype switching.
LOX	elastase and aggrecan accumulation in the ECM.
LOX-1	IL-1β production and ECM breakdown.

Footnote. MMP : matrix metalloproteinase*; TIMP* : tissue inhibitor of matrix metalloproteinase; NGAL : neutrophil gelatinase-associated lipocalin; ADAM : a disintegrin and metalloprotease; ADAMTS : a disintegrin and metalloproteinase with thrombospondin motifs; TSP: thrombospondin ; TN-C : Tenascin C; *CTGF/CCN2 :* Connective tissue growth factor/centralized coordination network 2; VSMCs : vascular smooth muscle cells; LOX : Lysyl oxidase; LOX-1 : lectin-type oxidized LDL receptor 1.

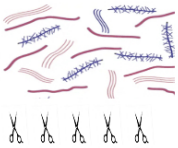
VSMCs alterations

Genetic Influence

FBN1
TGFBR1
TGFBR2
MMPs
TIMPs
COL3A1
ACT1
ACT2
MYLK
MYH11
LOX
PRKG1
SMAD3
PLAU
PSMA4
ERG
IL6R
CNN1
MYOCD
LMOD1
C1QB
C3AR1
VSIG4
5-LO
SMYD2
TCF7L2

+

ECM imbalance



MMPs
ADAMs
ADAMTSs
TIMPs
NGAL
TSPs
SIBILINGs
TNs
CCNs
SPARCs
Gla proteins
LOX
LOX-1

+

VSMCs alterations

Phenotype Switching
Migration
Apoptosis
EVs secretion

Other cellular and inflammatory alterations

Macrophage infiltration
PVAT alterations
VWRSCs anomalous differentiation
Cytokines demodulation

Arterial aneurysm